CLAIMS

1. A cell culture medium comprising a basal medium for animal or human cells to which is added: epidermal growth factor (EGF), an Rspondin, a fibroblast growth factor (FGF) and Nicotinamide.

2. The culture medium according to claim 1, wherein one, two, three, four or more from the group: a Wnt agonist, a BMP inhibitor, a TGF-beta inhibitor and HGF are added to the medium.

3. The culture medium according to claim 2, wherein

the BMP inhibitor is selected from Noggin, DAN, and DAN-like proteins including Cerberus and Gremlin;

the Wnt agonist is selected from one or more of Wnt, Wnt-3a, Norrin, and a GSK-inhibitor; and/or

the Rspondin is is selected from R-spondin 1, R-spondin 2, R-spondin 3 and R-spondin 4.

4. A cell culture medium which comprises or consists of EGF, FGF and/or HGF, a TGF-beta inhibitor and a Notch inhibitor.

5. A cell culture medium according to any one of the preceding claims, wherein

the Notch inhibitor is a gamma-secretase inhibitor, such as DAPT or dibenzazepine (DBZ) or benzodiazepine (BZ) or LY-411575;

the TGF-beta inhibitor is a small molecule inhibitor selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208, SJN 2511; and/or

the FGF is an FGF able to bind to FGFR2 or FGFR4 and is preferably FGF10.

6. A culture medium according to claims 4 or 5, wherein the medium additionally comprises Dexamethasone.

7. A culture medium according to any of the preceding claims, wherein one, two, three, four or more from the group gastrin, B27, N2, and N-Acetylcystein are added to the basal medium.

8. A method for obtaining and/or culturing a liver organoid, wherein said method comprises:

culturing epithelial stem cells from a liver fragment or a liver biliary duct in contact with an extracellular matrix in the presence of a medium, the medium comprising a basal medium for animal or human cells to which is added: EGF, an FGF and Nicotinamide.

9. A method according to any one of the preceding claims, wherein after at least one day, the medium is changed to a second medium.

10. A method according to claim 9, wherein the second medium is a second expansion medium comprising a basal medium to which is added: EGF, an FGF, and HGF, Nicotinamide and an Rspondin.

11. A method according to claim 9, wherein the second medium is a differentiation medium comprising a basal medium for animal or human cells to which is added EGF, FGF and/or HGF, and a Notch inhibitor.

12. A method according to claims 8, 10 or 11, wherein a TGF-beta inhibitor is additionally present in the medium and, optionally, wherein the wherein the TGF-beta inhibitor is a small molecule inhibitor selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208, SJN 2511.

13. A method according to any one of claims 9 to 12, wherein the method comprises culturing said epithelial stem cells in a cell culture medium comprising or consisting of a basal medium for animal or human cells to which is added EGF, a BMP inhibitor, R-spondin and Wnt; and subsequently in a second expansion medium which comprises or consists of a basal medium for animal or human cells to which is added: EGF, an FGF, and HGF, Nicotinamide, and an R-spondin; and subsequently in a differentiation medium which comprises or consists of a basal medium for animal or human cells to which is added: EGF, FGF and/or HGF, a TGF-beta inhibitor, and a Notch inhibitor.

14. A population of adult liver stem cells or a liver organoid, wherein the population of cells or liver organoid is obtainable by a method of any one of claims 1 to 13.

15. A liver organoid, which comprises a cystic structure and on the outside, a layer of cells with at least one bud and a central lumen.

16. A liver organoid according to any one of claims 14 to 15, which has one or more (e.g. 2, 3, 4 or all 5) of the following characteristics: (a) having a cell density of $>5\times10^5$

cells/cm^3, preferably >10×10^5 cells/cm^3; (b) having a thickness equivalent to 2-30 layers of cells, preferably a thickness equivalent to 2-15 layers of cells; (c) the cells mutually contact in three dimensions, (d) demonstrate a function inherent to healthy liver tissue, (e) have an elongated shape, with two defined domains.

17. A liver organoid according to any one of claims 14 to 16 comprising a single layered epithelial domain where polarized cells are detected and keratin markers are expressed and a second domain constituting the main body of the organoid and formed by a multilayered epithelia with non-polarized cells, optionally expressing albumin.

18. A liver organoid according to any one of claims 14 to 17, which originates from a cell expressing Lgr5, and which comprises a population of between at least 1x10^3 cells and 5x10^4, for example between at least 1x10^3 cells and 5x10^3 cells.

19. Use of a liver organoid as defined in any one of claims 14 to 18 in a drug discovery screen; toxicity assay; research of liver embryology, liver cell lineages, and differentiation pathways; gene expression studies including recombinant gene expression; research of mechanisms involved in liver injury and repair; research of inflammatory and infectious diseases of the liver; studies of pathogenetic mechanisms; or studies of mechanisms of liver cell transformation and aetiology of liver cancer.

20. A liver organoid or cell population according to any one of claims 14 to 18 for use in treating a liver disorder, condition or disease or for use in regenerative medicine.

CLAIMS

1. A method for obtaining a liver organoid by expanding epithelial stem cells, wherein said method comprises:

culturing at least one epithelial stem cell from a liver fragment or a liver biliary duct in contact with an extracellular matrix in the presence of a medium, the medium comprising a basal medium for animal or human cells to which is added: epidermal growth factor (EGF), a Wnt agonist, a fibroblast growth factor (FGF) and Nicotinamide.

2. A method according to claim 1, wherein one, two or three from the group: a bone morphogenetic protein (BMP) inhibitor, a TGF-beta inhibitor and human growth factor (HGF) are added to the medium.

3. A method according to claims 1 or 2, wherein after at least one day, the medium is changed to a second medium.

4. A method according to claim 3, wherein the second medium is a differentiation medium comprising a basal medium for animal or human cells to which is added EGF, FGF and/or HGF, a TGF-beta inhibitor and a Notch inhibitor.

5. A method according to any one of claims 1 to 4, wherein the method comprises culturing said epithelial stem cells in a cell culture medium comprising a basal medium for animal or human cells to which is added EGF, a BMP inhibitor, R-spondin and Wnt; and subsequently in a second expansion medium which comprises of a basal medium for animal or human cells to which is added: EGF, an FGF, and HGF, Nicotinamide, and an R-spondin; and subsequently in a differentiation medium which comprises or consists of a basal medium for animal or human cells to which is added: EGF, FGF and/or HGF, a TGF-beta inhibitor, and a Notch inhibitor.

6. A cell culture medium for expanding liver epithelial stem cells to obtain an organoid comprising a basal medium for animal or human cells to which is added: EGF, a Wnt agonist, an FGF and Nicotinamide.

7. The cell culture medium according to claim 6, wherein one, two or three from the group: a BMP inhibitor, a TGF-beta inhibitor and HGF are added to the medium.

8. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 6 or 7 wherein the Wnt agonist is selected from the group consisting of: a Wnt family member, R-spondin 1-4, Norrin, and a GSK-inhibitor.

9. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 7, wherein the TGF-beta inhibitor is a small molecule inhibitor selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208, SJN 2511.

10. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 6 or 7, wherein the FGF is an FGF able to bind to FGFR2 or FGFR4.

11. A method or a cell culture medium according to claim 10 wherein the FGF is FGF10.

12. A method or a cell culture medium according to any one of claims 1 to 5 or a cell culture medium according to claim 6 or 7, wherein one, two, three, four or more from the group gastrin, B27, N2, and N-Acetylcysteine are added to the basal medium.

13. A population of adult liver stem cells or a liver organoid, wherein the population of cells or liver organoid is obtained by a method of any one of claims 1 to 5 or 8 to 12.

14. A liver organoid obtainable by a method of any one of claims 1 to 5 or 8 to 12, which comprises a three dimensional cystic structure and on the outside, a layer of cells with a central lumen.

15. A liver organoid according to claims 13 or 14, which has one or more (e.g. 2, 3, 4 or all 5) of the following characteristics:

(a) having a cell density of $>5\times10^5$ cells/cm^3,

(b) having a thickness equivalent to 2-30 layers of cells,

(c) the cells mutually contact in three dimensions,

(d) demonstrate a function inherent to healthy liver tissue,

(e) have an elongated shape, with two defined domains,

(f) has at least one bud.

16. A liver organoid according to claim 15 wherein the organoid has a cell density of $>10 \times 10^5$ cells cm^3.

17. A liver organoid according to claim 15 wherein the organoid has a thickness equivalent to 2-15 layers of cells.

18. A liver organoid according to any one of claims 13 to 17 comprising a single layered epithelial domain where polarized cells are detected and keratin markers are

expressed and a second domain constituting the main body of the organoid and formed by a multilayered epithelia with non-polarized cells.

19. A liver organoid according to claim 18 wherein the second domain expresses albumin.

20. A liver organoid according to any one of claims 13 to 19, which originates from a cell expressing Lgr5, and which comprises a population of between at least $1x10^3$ cells and $5x10^4$ cells.

21. A liver organoid according to claim 20 wherein the organoid comprises a population of between at least $1x10^3$ cells and $5x10^3$ cells.

22. Use of a liver organoid as defined in any one of claims 13 to 21 in a drug discovery screen; toxicity assay; research of liver embryology, liver cell lineages, and differentiation pathways; gene expression studies including recombinant gene expression; research of mechanisms involved in liver injury and repair; research of inflammatory and infectious diseases of the liver; studies of pathogenetic mechanisms; or studies of mechanisms of liver cell transformation and aetiology of liver cancer.

23. Use of a liver organoid or cell population according to any one of claims 13 to 21 in the preparation of a medicament for treating a liver disorder, condition or disease or for use in regenerative medicine.